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Mice Selectively Bred for High- or Low-Alcohol-Induced Locomotion Exhibit Differences in Dopamine Neuron Function

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ABSTRACT

Elevated sensitivity to the euphoric or stimulant effects of ethanol is associated with higher levels of alcohol use in some human populations. Midbrain dopamine neurons are thought to be important mediators of both ethanol reward and locomotor stimulation. Patch-clamp recordings were used to examine the electrical properties of dopamine neurons in a genetic model of heightened (FAST) and reduced (SLOW) sensitivity to the locomotor-activating effects of ethanol. Pacemaker firing of dopamine neurons was faster in FAST than SLOW mice, as was the current density through I_H channels. Acute administration of ethanol accelerated the firing of dopamine neurons to a greater extent in recordings from FAST than SLOW mice. Dopamine neurons from FAST mice also exhibited reduced $GABA_A$ receptor-mediated synaptic input, compared with SLOW mice. The results suggest that dopamine neuron I_H channels, firing rate, and GABAergic input may play a role in sensitivity to the locomotor activation observed at early time points after ethanol administration and could underlie differences in sensitivity to alcohol relevant to risk for alcohol abuse.

Twin and adoption studies support a major genetic component in the predisposition to develop alcoholism, although the specific genes responsible have been elusive (Quickfall and el-Guebaly, 2006). Thus, endophenotypes, which are simpler, more easily measured traits that share a genetic relationship with alcoholism, are being used to investigate the genetic and neurobiological underpinnings of this complex disorder. Simple measures of ethanol sensitivity such as changes in balance and subjective stimulation (e.g., feeling "high" or energized) have shown promise as endophenotypes that predict susceptibility to excessive alcohol use (Schuckit and Smith, 2000; King et al., 2002). The biphasic effects of ethanol (stimulation followed by sedation) in humans are time- and dose-dependent (Addicott et al., 2007), and similar patterns of activity and sedation can be readily modeled in mice (Palmer et al., 2002). Such animal models allow research aimed at gaining a better understanding of the neurophysiological underpinnings of sensitivity to ethanol.

Ethanol has many established targets in the brain, and differential sensitivity of these targets could contribute to individual differences in alcohol-related behaviors. Dopamine neurotransmission originating in the midbrain is critical to the direct and conditioned reinforcing effects of ethanol (Bechtholt and Cunningham, 2005; Doyon et al., 2005) and ethanol-induced stimulation (Di Chiara and Imperato, 1988; Shen et al., 1995). Animals will self-administer ethanol directly into the posterior ventral tegmental area (VTA) (Rodd et al., 2004), and dopamine antagonists administered at the terminal site into the nucleus accumbens attenuate alcohol drinking (Samson et al., 1993). In vitro, acute administration of ethanol accelerates VTA and substantia nigra dopamine neuron firing (Brodie et al., 1999; Okamoto et al., 2006), which may contribute to increases in terminal dopamine release observed in vivo (Di Chiara and Imperato, 1988; Yim et al., 1998). GABA receptor-mediated inhibition of dopamine neurons (Tepper et al., 1998) may modulate the rewarding effects of ethanol (Samson et al., 1987; Melis et al., 2002). Furthermore, behavioral studies suggest that both $GABA_A$ and $GABA_B$ receptor subtypes play a role in the locomotor response to ethanol (Phillips et al., 1992; Boehm et

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ABBREVIATIONS: VTA, ventral tegmental area; MK-801, dizocilpine maleate; G protein-coupled inwardly rectifying potassium channel; mIPSC, miniature inhibitory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; IPSC, inhibitory postsynaptic current; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ZD 7288, 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyridinium chloride; CGP 56999a, [3-{[1-*R*-(3-carboxyphenyl)ethyl]amino}-2-(*S*)-hydroxy-propyl]-cyclohexyl-methyl-phosphonic acid.

al., 2002; Palmer et al., 2002; Holstein et al., 2009). Recent work by Okamoto et al. (2006) suggests that ethanol also enhances a nonselective cation conductance (I_H) in VTA and substantia nigra dopamine neurons. I_H facilitates spontaneous firing of dopamine neurons (Neuhoff et al., 2002; Puopolo et al., 2007; but see Mercuri et al., 1995), suggesting a cellular mechanism by which ethanol could directly modulate locomotion, reward, and other dopamine-related behaviors.

This investigation examined dopamine neurons in the substantia nigra of a genetic mouse model of heightened (FAST) and reduced (SLOW) sensitivity to the acute locomotor-stimulating effects of ethanol. The FAST and SLOW mice are the only lines that have been selectively bred for this ethanol sensitivity trait. The FAST mice also consume more ethanol than SLOW mice (Risinger et al., 1994) and exhibit reduced sensitivity to the sedative effects of ethanol (Phillips et al., 2002). These characteristics make them a compelling model of the cluster of traits associated with risk for alcoholism. The goal of the electrophysiological studies performed herein was to test the hypothesis that cellular mediators of dopamine neuron activity could mediate ethanol-induced stimulation. Dopamine neurons from FAST mice exhibited greater basal firing rate, greater I_H current density, and lower levels of GABAergic input. This genetic correlation between the electrophysiological findings and the ethanol sensitivity trait identify specific properties of dopamine neurons that may be controlled by the same genes that influence sensitivity to the stimulant effects of ethanol.

Materials and Methods

All animal protocols were approved by the institutional animal care and use committee at Oregon Health & Science University. Dopamine, ethanol, [Met]^5 enkephalin, MK-801, strychnine, 2,3-dihydroxy-6,7-dinitroquinoxaline, prazosin, and picrotoxin were obtained from Sigma-Aldrich (St. Louis, MO). ZD 7288 was purchased from Tocris Bioscience (Ellisville, MO). Baclofen, sulpiride, and hexamethonium were obtained from Sigma/RBI (Natick MA). CGP 56999a was a gift from Novartis (Basel, Switzerland).

Animals. Genetic selection of the FAST and SLOW mouse lines has been described previously (Phillips et al., 2002). In brief, mice from a heterogeneous stock (the product of an eight-way cross of genetically divergent inbred mouse strains) were tested for spontaneous locomotor activity for 4 min beginning 2 min after an injection of 2 g/kg ethanol i.p. Locomotor data after saline injection were collected at a 24-h interval from the ethanol response data, using the same procedure, and were subtracted from the ethanol response data to obtain an ethanol-induced stimulation score. For 37 generations, mice with high locomotor stimulation scores were selectively bred to each other and designated as FAST mice, whereas mice with low stimulation scores were interbred and designated as SLOW mice. Selection was subsequently relaxed (i.e., breeding was performed within line by arbitrary choice of breeders) because the additive genetic variance appeared to have been exhausted for several previous generations, suggesting that selection trait-relevant alleles had been homozygously fixed. In the approximately 50 generations hence, FAST and SLOW lines have been maintained without selection pressure but have retained a large phenotypic difference (Palmer et al., 2002). Second replicate FAST and SLOW mice from $S_{37}G_{75–93}$ (where S_{37} represents 37 selection generations and G_{XX} represents the total number of breeding generations) were used in these studies. Data were obtained from a total of 41 SLOW and 33 FAST alcohol-naive male mice. On most days, recordings were made from one FAST and one SLOW mouse and the order in which the animals were euthanized varied across days.

Brain Slice Electrophysiology. Mice (32–130 days old) were sacrificed under halothane anesthesia, and their brains were quickly removed and immediately placed into an ice-cold isotonic solution. Horizontal slices of the ventral midbrain (220 μm thick) were obtained with a vibrating microtome and stored in a physiological salt solution (containing 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 1.4 mM NaH_2PO_4, 25 mM $NaHCO_3$, 0.4 mM ascorbic acid, and 11 mM D-glucose) at 35°C for at least 30 min before recordings were initiated. Slices were perfused at a rate of 1.5 to 2.0 ml/min and maintained at 35°C throughout the experiment. Dopamine neurons of the substantia nigra were identified visually by their appearance and position in relation to the medial terminal nucleus of the accessory optic tract and electrophysiologically by their slow spontaneous pacemaker firing and the presence of the time-dependent hyperpolarization-induced cation conductance I_H. Substantia nigra neurons were chosen for this investigation because they have an established role in locomotion, respond to ethanol, and are more homogeneous than VTA neurons in their synaptic inputs and firing properties. Loose cell-attached patch-clamp experiments were performed with an internal pipette solution made up almost entirely of sodium HEPES plus 20 mM NaCl and 290 mOsm, pH 7.35. Whole-cell patch-clamp experiments were performed in voltage clamp at −60 mV. Experiments measuring GIRK-mediated currents used a pipette filling solution that contained 115 mM potassium methylsulfonate, 20 mM NaCl, 1.5 mM $MgCl_2$, 2 mM ATP, 0.2 mM GTP, 10 mM phosphocreatine, 10 mM potassium HEPES, and 10 mM BAPTA, pH 7.30 to 7.35, 268 to 275 mOsm. Experiments recording $GABA_A$-mediated currents used a pipette filling solution that contained 57.5 mM KCl, 57.5 mM potassium gluconate, 20 mM NaCl, 1.5 mM $MgCl_2$, 2 mM ATP, 0.2 mM GTP, 10 mM phosphocreatine, 10 mM potassium HEPES, and 10 mM BAPTA, 275 mOsm, pH 7.35. The synaptic blockers used throughout this study to isolate specific receptor systems were sulpiride (200 nM, dopamine D2), strychnine (10 μM, glycine), CGP 56999a (100 nM, $GABA_B$), picrotoxin (100 μM, $GABA_A$), MK-801 (10 μM, *N*-methyl-D-aspartate), 2,3-dihydroxy-6,7-dinitroquinoxaline (10 μM, AMPA), and prazosin (100 nM, α1 adrenergic). $GABA_B$ receptors were blocked during all experiments measuring $GABA_A$ receptor currents (see Ariwodola and Weiner, 2004).

Ethanol, [Met]^5 enkephalin, baclofen, and dopamine were applied by bath perfusion. Dopamine and GABA were also applied by iontophoresis. Iontophoretic pipettes were pulled from thin-wall glass to have a resistance of approximately 100 MΩ, then filled with 1 M dopamine or GABA. A backing current of −0.2 to −4.0 nA was applied to prevent passive leakage. The tip of the pipette was placed within 20 μm of the cell being recorded, and dopamine or GABA was ejected as a cation with a pulse of +190 nA for 1 to 5 s until the measured current reached a maximal plateau.

Synaptic currents were evoked by electrical stimulation with a bipolar platinum-stimulating electrode placed 50 to 150 μm caudal to the cell being recorded. $GABA_A$ currents were evoked with a single stimulation, and a train of five pulses was used to evoke $GABA_B$ and dopamine D2 receptor-mediated synaptic currents. The experimenter was blinded to the mouse line and the presence or absence of tetrodotoxin for the analysis of miniature and spontaneous $GABA_A$ inhibitory postsynaptic currents (mIPSCs and sIPSCs, respectively), which was done using Axograph 4.5.1 (John Clements). Cell capacitance and membrane resistance were also estimated by Axograph 4.5.1 in response to a 10-mV test pulse. Experiments were designed to be short so that virtually every cell recorded was included in the final analysis.

Statistical Analysis. Data were collected and quantified on a Macintosh G4 computer using Axograph 4.5.1 and Chart 4.0.1 (Molecular Devices, Sunnyvale, CA). One- and two-way analyses of variance were used to assess effects of independent variables, followed by Tukey's post hoc test, when appropriate. When recordings from FAST and SLOW mice were compared on single dependent measures, analyses were made with unpaired two-tailed Student's *t* tests. Statistical significance was defined a priori with $\alpha = 0.05$. Data

are presented as mean ± S.E., with *n* designating the number of cells obtained in each experimental group.

Results

Dopamine Neuron Spontaneous Firing Rate, Ethanol, and I_H. Dopamine neurons in the pars compacta of the substantia nigra were identified by their size, anatomical location, and width of their action potentials. Firing of these neurons was recorded using the loose cell-attached patch configuration. In acutely prepared brain slices, glutamate inputs are severed, and dopamine neurons fire almost exclusively in a rhythmic, pacemaker pattern. The spontaneous pacemaker firing rate of dopamine neurons was significantly higher in neurons obtained from FAST mice (1.93 ± 0.08 Hz) than those obtained from SLOW mice (1.41 ± 0.09 Hz, Fig. 1). In addition, a number of neurons from SLOW mice were firing in couplets rather than in a pacemaker pattern, which was not the case in FAST mice. These neurons were not included in the analysis of firing rate.

Dopamine neuron firing rate and pattern are critical to the behavioral consequences of terminal dopamine release. One determinant of spontaneous firing rate in dopamine neurons is a hyperpolarization-induced, nonselective cation conductance termed I_H (Puopolo et al., 2007), recently identified as a putative target of ethanol action (Okamoto et al., 2006). Results obtained with a 50-mV hyperpolarization for 1 s using whole-cell patch clamp indicated that dopamine neurons from FAST mice had a significantly higher I_H current density than those obtained from SLOW mice (Fig. 2). This was a combined effect of slightly larger raw I_H values from FAST mice (441 ± 33 versus 400 ± 38 pA, $P = 0.41$), with significantly smaller membrane capacitance values (31.6 ± 1.1 versus 36.3 ± 1.1 pF, $P = 0.004$). Dopamine neurons from FAST mice also exhibited significantly higher input resistance (239 ± 24 versus 180 ± 9.8 MΩ, $n = 39–43$, $P = 0.03$, data not shown). When the dopamine neuron firing rate was once again directly measured in the loose cell-attached configuration, blockade of I_H with 30 μM ZD 7288 eliminated the line difference in the dopamine neuron firing rate (Fig. 2C). This concentration of ZD 7288 has been shown previously to produce complete blockade of I_H in dopamine neurons from C57BL/6J mice (Neuhoff et al., 2002).

Acutely, ethanol directly accelerates dopamine neuron firing (Brodie et al., 1999) and also increases I_H in a cAMP-dependent manner (Okamoto et al., 2006). If I_H is an important determinant of initial locomotor response to ethanol, ethanol-induced firing may differ between the FAST and SLOW lines. Consistent with previous reports, bath perfusion of a pharmacologically relevant concentration of ethanol (50–80 mM; Harris et al., 2008) modestly increased spontaneous firing of dopamine neurons (Fig. 3). However, this increase was significantly more pronounced in dopamine neurons from FAST animals. Taken together, the results suggest that I_H-induced firing of dopamine neurons is genetically correlated with the selection trait and provide evidence that I_H could contribute to the acute locomotor response to ethanol.

No Differences Observed in GIRK Channel-Mediated Conductances. A second determinant of firing rate that may have been affected by selection of the FAST and SLOW mice is potassium signaling via dopamine D2- and $GABA_B$ receptor activation. Neurotransmission through these receptors activates an inhibitory G-protein coupled potassium conductance (GIRK) that has direct effects on dopamine neuron firing (Beckstead et al., 2004). This GIRK conductance has also been identified as a potential direct target of ethanol action in the brain (McDaid et al., 2008). Thus, GIRK-mediated conductances were examined in dopamine neurons from FAST and SLOW mice. Recordings using whole-cell patch clamp suggested that there was no line difference in currents obtained by synaptic activation of $GABA_B$ or D2 receptors or in maximal currents elicited by pharmacological activation of these receptors with exogenously applied agonists (Fig. 4). This provides evidence that GIRK channels in dopamine

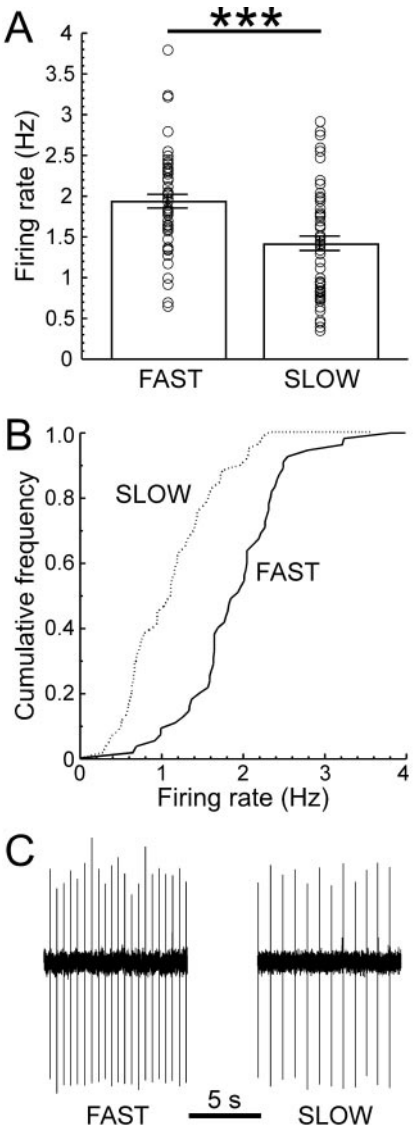

Fig. 1. Dopamine neurons from FAST mice exhibit a more rapid spontaneous firing rate. Midbrain dopamine neurons exhibit a tonic, regular firing pattern when observed in vitro in brain slice preparations. Examination of firing rate using the loose patch cell-attached configuration revealed that the dopamine cell basal firing rate is significantly faster in slices from FAST compared with SLOW mice [A and B, $t_{(113)} = 4.35$, $P < 0.0001$, $n = 55–60$ cells from 17–18 mice per group]. Sample recordings in slices taken from a FAST and a SLOW mouse illustrate the difference in pacemaker firing rate (C). The data presented are predominantly from an independent experiment but do include neurons also presented in Figs. 2C and 3A.

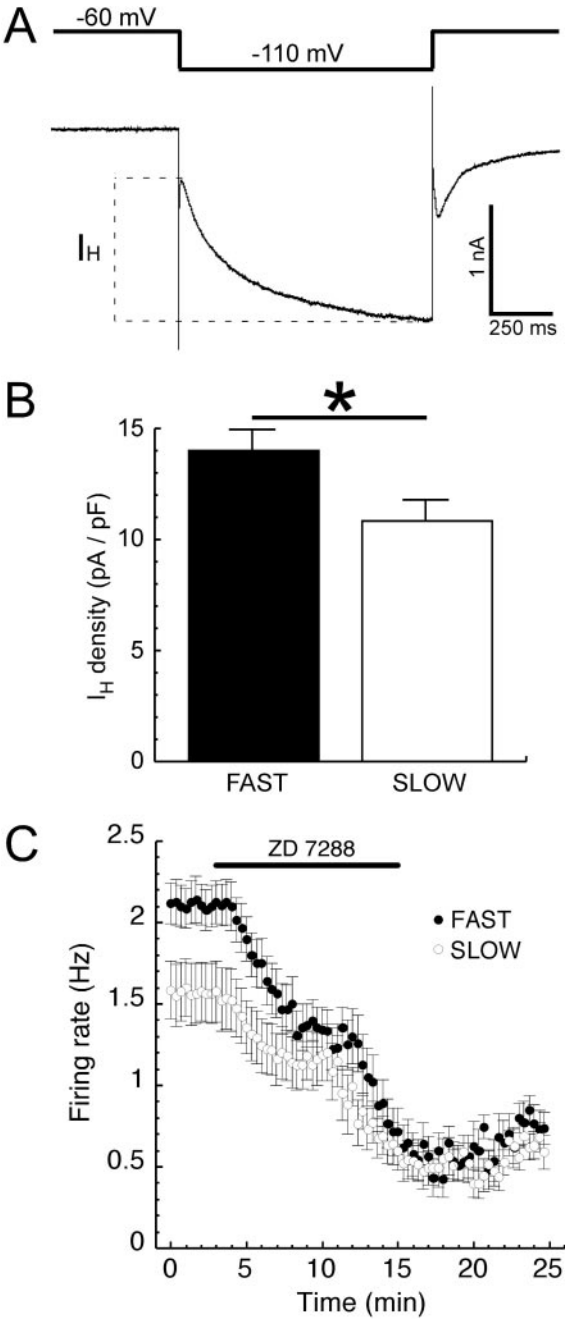

Fig. 2. Dopamine neurons from FAST mice exhibit a higher I_H current density. The nonselective cation conductance I_H was examined in voltage clamp with a hyperpolarization from −60 to −110 mV, measured as the time-dependent component illustrated by the dotted lines (A). Measured as a function of cell capacitance, dopamine neurons from FAST mice exhibited a higher I_H density compared with those from SLOW mice [B, $t_{(67)} = 2.37$, $P = 0.02$, $n = 33–36$ cells from six mice per group], which could contribute to their faster firing rate. When I_H channels were blocked with ZD 7288 (30 μM), spontaneous firing was more substantially slowed in cells from FAST mice [C, $t_{(22)} = 2.53$, $P = 0.019$, $n = 12$ cells from four to six mice per group].

neurons are not involved in the early locomotor response to ethanol and probably do not contribute to the differences observed in cell firing.

Line Differences in $GABA_A$ Receptor-Mediated Transmission. $GABA_A$ receptors in the midbrain have been implicated in the neuronal effects of ethanol (Samson et al., 1987; Melis et al., 2002). If dopamine neuron firing is involved in ethanol-induced locomotor activation, then FAST and SLOW mice may exhibit differential $GABA_A$ receptor-mediated input onto dopamine neurons. $GABA_B$, glycine, and ionotropic glutamate receptors were pharmacologically antagonized, dopamine neurons were patch clamped, and $GABA_A$ receptor-mediated currents were evoked electrically. The amplitude of $GABA_A$ inhibitory postsynaptic currents (IPSCs) was significantly larger in dopamine neurons obtained from SLOW mice (Fig. 5). Maximal $GABA_A$ receptor-mediated currents activated by iontophoresis of exogenous GABA were also larger in slices from SLOW mice, suggesting a postsynaptic component for this effect. The μ opioid receptor agonist [Met]^5 enkephalin is known to presynaptically inhibit GABA release onto dopamine neurons (Johnson and North, 1992). The effect of [Met]^5 enkephalin on evoked

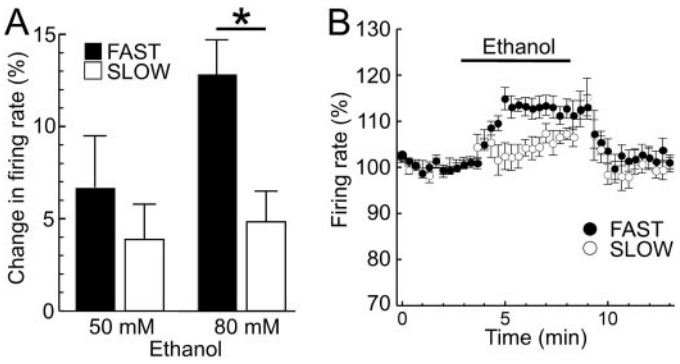

Fig. 3. Ethanol increases the dopamine neuron firing rate to a greater extent in recordings from FAST mice. Ethanol (50–80 mM) produced a subtle increase in pacemaker firing of dopamine neurons (A). This increase was significantly larger in recordings from FAST mice [$F_{(1,38)} = 6.84$, $P = 0.013$ for main effect of line, $n = 9–11$ cells from four mice per group]. The time course of the effect of ethanol (80 mM) is illustrated in B ($P = 0.035$, Tukey's post hoc test for 80 mM ethanol applications between recordings from FAST versus SLOW mice).

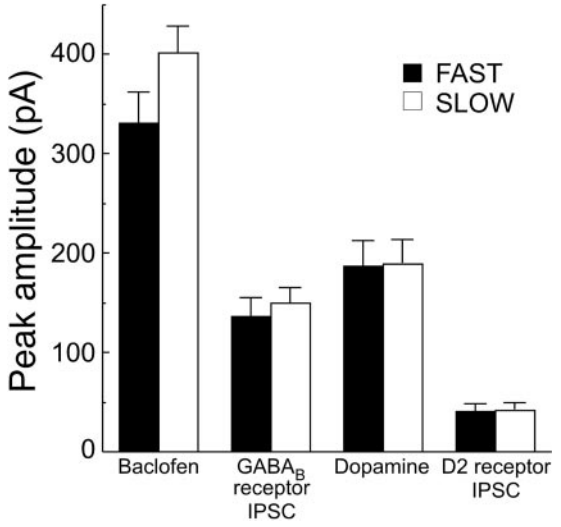

Fig. 4. GIRK channel-mediated inhibitory transmission does not differ between FAST and SLOW mice. Experiments using whole-cell patch clamp of dopamine neurons revealed no difference in GIRK channel-mediated currents in slices obtained from FAST and SLOW mice. GIRK conductance was examined through activation of metabotropic $GABA_B$ and dopamine D2 receptors, both through application of exogenous agonists (30 μM baclofen and dopamine applied through iontophoresis) and by evoking IPSCs in the presence of synaptic blockers ($n = 9–14$ cells from three to six mice per group; baclofen, $P = 0.095$; $GABA_B$ IPSC, $P = 0.59$; dopamine, $P = 0.87$; D2 IPSC, $P = 0.92$).

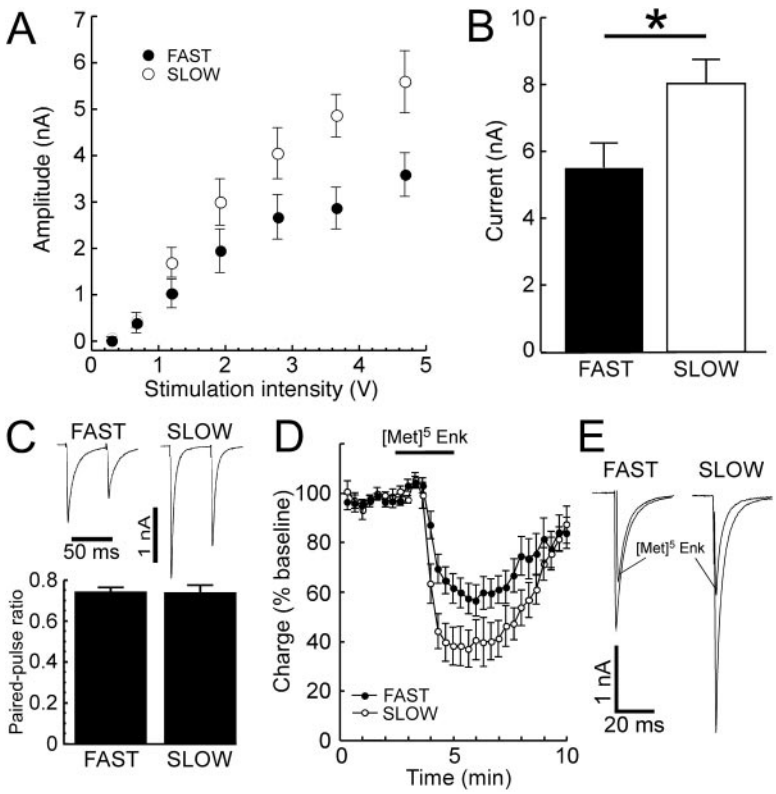

Fig. 5. $GABA_A$ receptor input to dopamine neurons is larger in recordings from SLOW mice. Endogenous GABA release was evoked with a bipolar-stimulating electrode placed caudal to the dopamine neuron being recorded. A preliminary investigation found that the amplitude of the inhibitory synaptic current was significantly larger in slices obtained from SLOW compared with FAST mice ($P = 0.011$, $n = 30–33$ cells from six to seven mice in each group, data not shown). Full stimulus-response curves were subsequently constructed with varying stimulus intensities (A), again demonstrating significantly larger IPSCs in recordings made from SLOW mice [$F_{(1,18)} = 36.7$, $P = 0.038$ for main effect of line; $F_{(6,108)} = 4.71$, $P = 0.0003$ for line-stimulus interaction, $n = 9–11$ cells from three mice per group]. $GABA_A$ receptor-mediated currents were also larger in recordings from SLOW mice (B) when activated with iontophoresis of exogenous GABA [1 M, $t_{(20)} = 2.44$, $P = 0.024$, $n = 11$ cells from three mice per group]. There was no difference in the paired-pulse ratio of evoked $GABA_A$ receptor currents in brain slices taken from FAST versus SLOW mice (C). Care was taken while conducting the experiments for data shown in A to C to use identical slice configuration, stimulator depth and position, and iontophoretic pipette position relative to each neuron being recorded. Next, GABA release was presynaptically inhibited by activation of μ opioid receptors with the agonist $[Met]^5$ enkephalin (10 μM). The inhibition produced by enkephalin was significantly larger in recordings from SLOW mice [D and E, main effect of line, $F_{(1,20)} = 4.76$; $P = 0.041$, $n = 10–12$ cells from three mice per group].

$GABA_A$ IPSCs was significantly larger in recordings obtained from SLOW mice, suggesting that alterations in the presynaptic release of GABA onto dopamine neurons may also be controlled by the same genes responsible for ethanol-induced locomotor activation. However, no difference in paired-pulse ratio was observed between recordings from FAST and SLOW mice (Fig. 5C), indicating that probability of release was not affected by genetic selection.

To further investigate these actions, the release of GABA was studied in the absence (sIPSCs) or the presence (mIPSCs) of tetrodotoxin, which eliminates action potentials by blocking voltage-gated sodium channels. There was substantially greater frequency of $GABA_A$ receptor-mediated sIPSCs in recordings obtained from SLOW animals (Fig. 6). The sIPSCs also exhibited larger amplitudes in recordings obtained from SLOW mice. These effects were eliminated in the presence of tetrodotoxin, suggesting that line differences in spontaneous GABAergic transmission are action potential dependent. Perfusion of the $GABA_A$ receptor antagonist picrotoxin (100 μM) did not affect dopamine neuron firing rate in brain slices taken from SLOW mice (+0.09 ± 1.74%, $n = 7$ cells from three mice, data not shown). Thus, differences in $GABA_A$ receptor-mediated input are probably not responsible for the observed differences in pacemaker firing (e.g., Fig. 1).

Ethanol acutely enhances GABA transmission in dopamine neurons (Melis et al., 2002). To investigate the contribution of this effect on initial stimulation, GABA transmission was studied in the absence and presence of ethanol (80 mM). Although ethanol did increase the amplitude and the area under the curve of evoked $GABA_A$ IPSCs, there was no difference in this effect between dopamine neurons obtained from FAST and SLOW mice (Fig. 7A). Furthermore, ethanol did not have an obvious effect on the frequency of sIPSCs in FAST or SLOW mice (Fig. 7B) or on their amplitude (data not shown).

Discussion

Alcohol is very commonly abused, and a multitude of targets have been identified as putative mediators of its central effects. This investigation sought not to identify new targets but to use the combination of electrophysiology and a behavioral genetic tool to identify which previously identified targets could be determinants of a specific ethanol-related behavior: locomotor stimulation. Ethanol-induced locomotor stimulation can be easily measured in mice and involves some of the same neuroanatomical pathways shown to influence the rewarding properties of drugs of abuse (Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Boehm et al., 2002). Moreover, elevated initial sensitivity to the euphoric or stimulatory effects of ethanol is associated with higher levels of alcohol consumption and may be a predictor of the propensity to develop alcoholism in some individuals (King et al., 2002; Gabbay, 2005). The FAST and SLOW mouse lines were selectively bred for differential sensitivity to locomotor activation at an early time point after a single injection of ethanol. Much of the research using these lines has attempted to identify the neurophysiological and neurochemical substrates important for the initial sensitivity difference by looking for pleiotropic effects of the genes involved (i.e., genetic correlation). Measurable differences between the FAST and SLOW lines for alternative (nonselected) traits would suggest that common alleles influence both traits. The present study describes the first electrophysiological analysis of the FAST and SLOW lines and the first attempt to look for differences in the synaptic inputs to specific neurons in these mice. Results using this combination of electrophysiological,

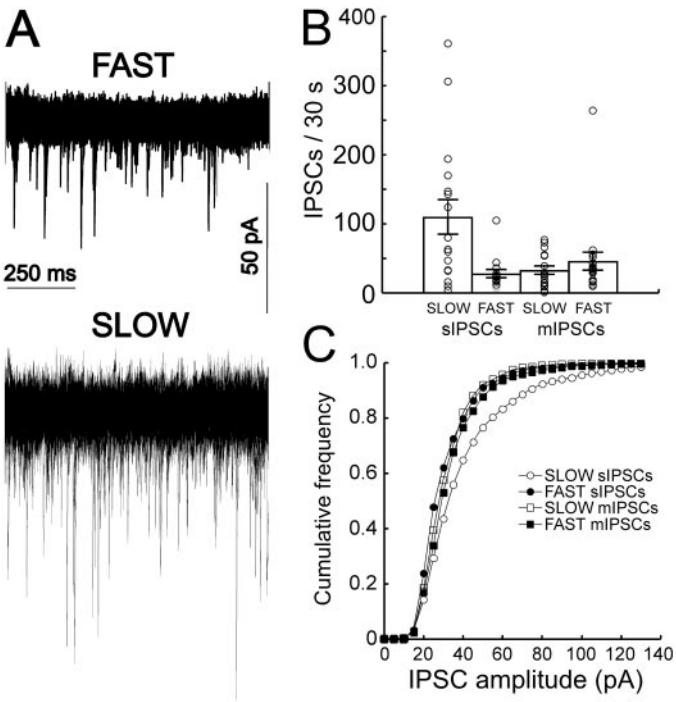

Fig. 6. Spontaneous $GABA_A$ IPSCs are larger and more frequent in SLOW mice. In the presence of pharmacological blockers of glycine, *N*-methyl-D-aspartate, AMPA, and $GABA_B$ receptors, spontaneous and miniature $GABA_A$ IPSCs were recorded in dopamine neurons of slices from FAST and SLOW mice. Tonic GABAergic input was more pronounced in cells from SLOW mice (A, 30 1-s sweeps overlaid). SLOW mice exhibited a greater frequency of sIPSCs (B), and this effect was blocked in the presence of tetrodotoxin (300 nM, mIPSCs). sIPSCs from SLOW mice also exhibited a larger amplitude (C) (n = 15–20 cells from three to four mice per group, Tukey's post hoc test, $P = 0.02$ for differences in frequency between SLOW sIPSCs and the other groups, $P < 0.0001$ for differences in amplitude).

behavioral, and genetic tools suggest that dopamine neuron firing rate, I_H current density, and $GABA_A$ receptor-dependent input could be controlled by some of the same genes responsible for differences in sensitivity to ethanol-induced locomotor activation.

I_H-Dependent Firing of Dopamine Neurons. Dopamine neurons in the ventral midbrain were examined in this study because of their dual role in locomotion and ethanol-related reinforcement (Wise and Bozarth, 1987; Samson et al., 1993; Shen et al., 1995; Rodd et al., 2004; Bechtholt and Cunningham, 2005). FAST mice exhibit stronger preference for ethanol than SLOW mice (Risinger et al., 1994), and behavioral studies suggest that dopaminergic mechanisms are important for the expression of ethanol-induced locomotor stimulation in FAST mice (Shen et al., 1995). FAST mice are more sensitive than SLOW mice to the locomotor stimulant effects of drugs that increase extracellular dopamine (Meyer et al., 2009). As measured with in vivo microdialysis, extracellular dopamine levels are increased in response to most drugs of abuse, including ethanol (Di Chiara and Imperato, 1988; Yim et al., 1998), and FAST mice show a greater dopamine response than SLOW mice after both ethanol and cocaine treatment (Meyer et al., 2009). This ethanol-induced increase is blunted in dopamine D2 receptor knockout mice (Job et al., 2006), which also exhibit reduced ethanol-induced conditioned place preference (Cunningham et al., 2000), decreased ethanol preference (Phillips et al., 1998), and decreased operant responding for ethanol (Risinger et al., 2000). However, D2 receptor knockout mice also display heightened ethanol-induced locomotor stimulation (Palmer et al., 2003), opposite to what would be predicted from the dopamine and reward data, suggesting that the relationships among these traits are not completely straightforward and may be dependent upon genetic background. The preponderance of evidence that dopaminergic mechanisms contribute to the neuropharmacological effects of ethanol suggests that the correlated endophenotypes identified herein are not related to the accidental fixation of selection trait-irrelevant genes. Rather, they suggest the pleiotropic influence of genes on sensitivity to ethanol-induced stimulation and dopamine-mediated mechanisms.

Acutely, ethanol produces a modest but reproducible increase in dopamine neuron firing (Brodie et al., 1999). This effect may be partially dependent on I_H channels, which increase dopamine neuron firing and have also been identified in mice (but not rats; see McDaid et al., 2008) as being an important target of ethanol action (Okamoto et al., 2006). The observation that dopamine neuron spontaneous firing rate is higher in FAST than SLOW mice suggests that firing may be an important determinant of the initial stimulation to ethanol. The higher spontaneous firing rate is also consistent with the slightly higher basal locomotion that has been observed sometimes in FAST mice (Holstein et al., 2005). A line difference was also observed in I_H current density, offering a plausible mechanism by which firing could be altered. Blocking I_H with ZD 7288 eliminated the line difference in firing rate, and acute administration of ethanol increased the firing rate to a greater extent in FAST than SLOW mice. It is

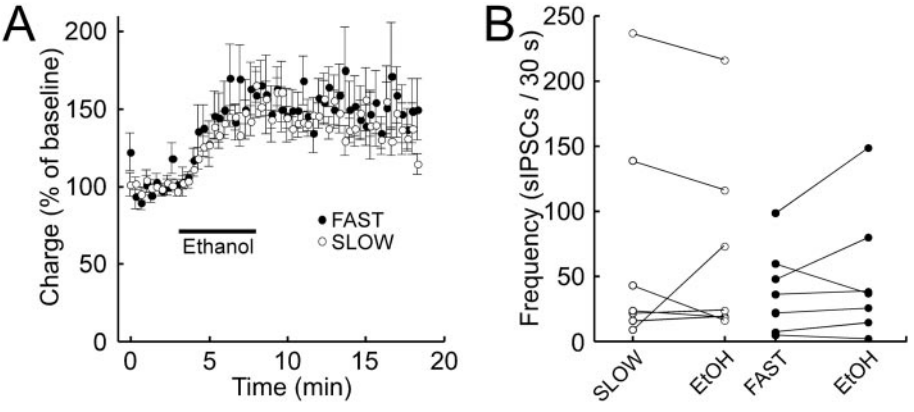

Fig. 7. Ethanol effects on GABAergic input to dopamine neurons do not exhibit a line difference. $GABA_A$ receptor-mediated IPSCs were evoked in midbrain dopamine neurons. Bath perfusion of ethanol (80 mM) increased the area under the curve (A) of the IPSCs. However, there was no difference between the FAST and SLOW lines [n = 14 cells from six to seven mice per group, main effect of line, $F_{(1,25)} = 0.0025$, $P = 0.96$]. Bath perfusion of ethanol had no obvious effects on the frequency (B) or amplitude (data not shown) of sIPSCs recorded from FAST and SLOW mice (n = 7 cells from three mice per group).

impossible to say for certain what effect a subtle increase in firing observed in vitro would have in vivo, where firing patterns are much more complex. However, 2 g/kg ethanol induces locomotion, despite reports that similar concentrations produce merely a ~10% increase in firing rate in vitro (Okamoto et al., 2006) and a 30% increase in nucleus accumbens dopamine in FAST mice in vivo (Meyer et al., 2009). Taken together with the line difference in behavior, these differential acute effects provide evidence that I_H-induced dopamine neuron firing could contribute to ethanol-induced locomotion.

GABA Receptor-Mediated Transmission. Behavioral studies performed on FAST and SLOW mice implicate $GABA_A$ receptor signaling in ethanol-induced locomotor activation (Phillips et al., 1992; Palmer et al., 2002). Systemically administered $GABA_A$ receptor ligands have differential effects on FAST and SLOW mice (Shen et al., 1998; Palmer et al., 2002), and differential sensitivities to barbiturates and benzodiazepines were among the first correlated traits identified during selection (Phillips et al., 1992). The present study provides additional evidence that $GABA_A$ receptor signaling is important to initial locomotor stimulation and identifies dopamine neurons as one potential locus of this effect. Acute administration of ethanol equivocally increased the amplitude of evoked $GABA_A$ IPSCs in recordings from both FAST and SLOW mice. The observation that acute ethanol had no differential line effect argues against a line difference in subunit composition, as does the lack of a line difference in IPSC kinetics (data not shown).

SLOW mice did exhibit a larger basal frequency and amplitude of spontaneous $GABA_A$ IPSCs, an effect that was eliminated when voltage-sensitive sodium channels were blocked with tetrodotoxin. However, because the frequency of $GABA_A$ sIPSCs was unaffected by acute ethanol, it is unlikely that release probability is critical to initial locomotor stimulation. This assertion is bolstered by the absence of a line difference in paired-pulse ratio of evoked $GABA_A$ IPSCs. Both the frequency and amplitude of sIPSCs were higher in recordings from SLOW mice, typically interpreted as dual pre- and postsynaptic effects. However, because there was no difference between sIPSCs from FAST mice and mIPSCs from either line, it is more likely that this form of spontaneous transmission is simply absent in FAST mice. Regardless of the anatomical and physiological factors involved, the present results suggest that the genes that control GABAergic input to dopamine neurons could also be determinants of initial sensitivity to ethanol. Given the dual importance of dopamine neurons in both ethanol reinforcement and locomotor activation, individual variation in $GABA_A$ transmission probably contributes to the propensity for higher levels of ethanol use.

Previous evidence suggests that $GABA_B$ receptors are involved in ethanol-induced locomotor stimulation. When administered systemically or into the posterior region of the VTA, the $GABA_B$ receptor agonist baclofen blocks ethanol stimulation in FAST mice (Shen et al., 1998; Boehm et al., 2002), although there is no line difference in $GABA_B$ receptor density in either the substantia nigra or VTA (Boehm et al., 2002). No difference in $GABA_B$ receptor signaling was observed presently, whether receptors were activated synaptically or through the exogenous application of baclofen. There was also no observable difference in dopamine D2 receptor signaling in these neurons, suggesting that GIRK channels in the substantia nigra are not critical to ethanol-induced activation at early time points. Dopamine D2 receptor-mediated IPSCs are also not affected by acute administration of 80 mM ethanol (our unpublished observation).

In summary, the current results provide evidence that dopamine neurons are critical to the genetically determined difference in sensitivity to the initial locomotor response to ethanol. This is the first evidence that two previously identified targets of ethanol action, the nonselective cation conductance I_H and dopamine neuron firing rate (Brodie et al., 1999; Okamoto et al., 2006), could be determinants of the initial locomotor response. These findings also affirm that $GABA_A$ receptor-mediated transmission modulates this initial stimulation and suggest that one potential locus of this effect is in the substantia nigra. The results presented herein are correlational and would be bolstered by replication in future studies using the other replicate set of FAST and SLOW lines. However, given the previously identified role of dopamine in reward and locomotion and the multiple distinct effects observed presently on the same neurons, it is reasonable to conclude that dopamine neurons play a part in mediating locomotor activation after ethanol administration. Ethanol-induced locomotor stimulation is a complex trait that involves many neurophysiological substrates. Because genetic differences in initial sensitivity to ethanol may have predictive value for the risk to develop alcoholism, the correlated neurophysiological pathways identified herein could be legitimate candidate targets for alcoholism treatment regimens.

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